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13. Abstract ***(Maximum 200 Words)*** ***(abstract should contain no proprietary or confidential information)***

This project is evaluating whether oral contraceptives and parity are as protective against ovarian cancer in BRCA1/2 carriers as they are for women in general. It is also determining whether there are survival differences between BRCA1/2 carriers with ovarian cancer compared to women with sporadic disease. The study employs a case-case design. We will identify about 400 Jewish women with epithelial ovarian cancer. We will genotype these women for the 3 BRCA1/2 mutations found in Ashkenazi women. We will then compare oral contraceptive use and parity between carriers and non-carriers. We will also compare survival differences between the two groups. In the second year of the project, we identified 242 Jewish women with invasive epithelial ovarian cancer for whom *BRCA1/2* genotyping data was available or was obtained by our assay. We then compared OC use and reproductive factors for those women with and without a mutation. Initial results suggest that OC use, childbearing and breastfeeding are as protective in *BRCA1/2* carriers as they are in women in general. A manuscript detailing these findings is under review.

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INTRODUCTION:

The goal of this project is to determine whether oral contraceptives (OCs) and parity are as protective against ovarian cancer in BRCA1/2 carriers as they are for women in general. The second goal is to determine whether there are survival differences between BRCA1/2 carriers with ovarian cancer compared to women with sporadic disease. The study employs a case-case design. We will identify about 400 Jewish women with epithelial ovarian cancer. We will genotype these women for the 3 BRCA1/2 mutations found in Ashkenazi women. We will then compare oral contraceptive use and parity between carriers and non-carriers. We will also compare survival differences between the two groups.

BODY:

In this section, we describe our accomplishments according to the Work Plan originally approved. .

Task 2 Performance of Laboratory Assays, Months 1-28:

a. Specimens (400) will be located, cut, labeled with the new study ID and shipped to the core lab

b. Assays (400) to detect BRCA1/2 mutations will be performed and the results recorded on study forms

c. A subset of specimens (80) will be retested to validate the laboratory results

Specimen Retrieval:

We originally proposed to identify 400 specimens from a combination of two sources:

a). A parent study that housed an estimated 75 specimens at 35 hospitals in Eastern Pennsylvania

b). A series of hospitals in Pittsburgh, Cleveland and NY that housed an additional 300+ specimens

We began employing this approach, but found it very time consuming. We spent many months on hold as we attempted to execute several IRBs. Some IRBs required that we obtain patient consent to retrieve their tissue specimens and perform a chart review. We were not able to accommodate this request and had to drop any site requesting this consent. Note, as originally proposed, because we are obtaining tissue specimens and performing chart review in a blinded fashion, our protocol meets the federal regulations for exempt status (45CFR 46.101b4).

Accordingly, we have amended our approach to be more efficient. First, we identified 3 hospitals that together house approximately 400 eligible specimens. These include: North Shore University Hospital (289 specimens), Long Island Jewish Hospital(88 specimens) and Northwestern University Hospital (101 specimens). IRB approval for the study at the first two sites was obtained year 1. IRB approval for Northwestern University Hospital was obtained in May 2002. A copy of that letter appears in Appendix A.

We began obtaining specimens from the NY sites. In September 2002, we successfully obtained 34 specimens from North Shore University Hospital. Specimen retrieval includes a block of tumor tissue, a block of normal tissue, a slide of the tumor and a slide of the normal tissue. A pathology report accompanies all specimens. Once the specimens and accompanying path report is received, Dr. Naus (the study pathologist in Pittsburgh) reviews the slides and report to confirm eligibility of the specimens for the study. Eligibility criteria include: diagnosis of invasive epithelial ovarian cancer between Jan 1, 1990 and Dec 31 1999. To date, all specimens have met our criteria.

Dr. Naus also confirms the histological subtype of the tumor as well as tumor grade using the slides and data sent from the sites. Thus far, we have had no discrepancies between the original pathology report and Dr. Naus' review.

We are requesting both tumor and normal tissue for two reasons. First, we will do our genotyping on the normal tissue to ensure we truly identify a germline mutation in the BRCA/2 genes. Second, we are banking the tumor and normal tissue in anticipation of future research. For example, we intend to look at mechanisms of BRCA inactivation (such as LOH and methylation) as they affect response to chemotherapy and survival. Thus, our efforts in this project are also supporting future research.

Laboratory Assays:
We tested our laboratory assays on a sample of 36 specimens that we had obtained year 1 from the Pennsylvania hospitals. The laboratory assays are performed in the laboratory of Dr. Jeffrey Kant in the Division of Molecular Diagnostics at the University of Pittsburgh Medical Center. The Division currently performs clinical BRCA1/2 screening for the Ashkenazic Jewish mutation panel using allele-specific hybridization (ASO) and direct DNA sequencing approaches. These assays employ polymerase chain reaction (PCR) amplification in which several gene regions are co-amplified, spotted and hybridized to wild type and mutant probes, and detected by chemiluminescence. Samples testing positive for a mutation are confirmed by DNA sequence analysis using an ABI Model 373 semi-automated DNA sequencer.

For quality control purposes, a subset of 8 specimens were sent to the laboratory in a blinded duplicate fashion. The findings for the 8 specimens were concordant, providing confidence in the laboratory quality. For quality control purposes, we have decided to batch the genotyping of the remaining specimens during year 3 of the study. Thus, we have not done any additional genotyping.

DNA for this study is obtained from paraffin sections. Two 5-10 micron tissue sections are processed. Excess paraffin is removed by razor blade. The sections are dewaxed in xylene, washed in ethanol, and suspended in a Tris-EDTA-Triton X-100 extraction buffer. An overnight proteinase K digestion is then performed at 56 degrees Centigrade. The proteinase K is heat-killed by boiling. Finally, the solution is pelleted at high speed, and the extract saved for subsequent analysis. A commercial kit method (Puregene by Gentra) has also worked successfully in our hands. To ensure that we identify germline mutations (not somatic) wherever possible we isolate DNA from the normal tissue block. Thirty two of the 34 specimens from NY contained both a tumor and normal block; only 2 contained only a tumor block. Dr. Stanek informed us that he was unable to identify a normal block on those two women.

Exons 2 and 20 of BRCA1 as well as the portion of BRCA2 exon 11 containing nucleotide 6174 are amplified by a 35-cycle multiplex polymerase chain reaction. Amplicons are then denatured, filtered onto positively-charged nylon support substrates, and hybridized to separate oligonucleotide probes (5'-conjugated with alkaline phosphatase) that detect wild type and mutant sequences. Heterozygous controls are included for reactions as well as minus-DNA blanks. Following hybridization and washing, blots are developed with the chemiluminescent substrate, Lumi-Phos. Results are scored by visual inspection of autoradiograms after brief exposure.

Problems encountered and measures taken:

The major problems we have encountered include

1). Obtaining IRB approval for this study at participating sites
2). Obtaining specimens from the approved sites

To address problem 1, we reduced the number of sites we are including in this study and have now obtained the necessary IRBs.

To address problem 2, we are working more closely with the investigators at the three sites. We acknowledge that the New York hospital sites have been delayed in sending us specimens. This was due to a change in research team members at that site. That change resulted in the project being put on hold for almost a year. A new pathologist at that site has been obtained (Dr. Stanek), and we have begun once again to work with the investigators to move the project forward. We feel confident in Drs. Menzin and Stanek's commitment to the project and anticipate its successful completion.

We have been working with Dr. David Fishman at Northwestern University. Since the IRB was approved in May, we have identified the 101 eligible specimens and are working with his group to perform the necessary chart reviews and obtain the pathology specimens.

Task 4 Abstraction of Medical Records, Months 12-24:

a. *Medical record abstractions will be performed for all 400 subjects*
b. *Medical record data (400 subjects) will be reviewed by Dr. Edwards*
c. *Pathology slides (400 subjects) will be reviewed by Dr. Naus*
d. *Data entry and quality control measures will be ongoing*

In year two we identified two clinical staff persons to perform our chart review. One was a nurse at the NY site. The other is a clinical geneticist at the Northwestern site. All chart reviews are reviewed by Dr. Edwards (the study Gynecologic Oncologist) for quality control. Unfortunately, the chart reviews received from the NY site failed to meet our quality levels. Accordingly, we have identified a new individual in NY to perform the chart reviews. We have recently hired an oncology fellow(Dr. Meghna DeSaui) to begin performing the chart reviews at the NY site. AS of September 24. 2002, Dr. DeSaui completed 6 charts reviews, which Dr. Edwards will review. If Dr. Edwards is satisfied with the quality of the work, we will proceed with the remaining chart reviews at NY using Dr. DeSaui as the abstractor.

We are working with Dr. Fishman's genetic assistant to perform the chart reviews at Northwestern University.

We have not begun data entry because our quality control on the data abstraction found several problems (as described above). We will continue to apply strict quality control standards to ensure the correctness of the data.

Problems encountered and measures taken:
As discussed, we encountered problems with the chart reviews at one site. These problems were uncovered during our quality control reviews. We replaced the staff person with a new person and anticipate the successful abstraction of the necessary data from our charts.

Note that we have decided to only undertake chart reviews on women for whom we obtain pathology specimens, since the chart review data is not relevant without the genotyping data.

KEY RESEARCH ACCOMPLISHMENTS:

Independent of the proposed approach to address the two specific aims of the project, we identified a series of 242 Ashkenazi Jewish women with invasive epithelial ovarian cancer. These women had participated in two population-based case control studies, a genetic counseling center at Northwestern University and a multi-center study of Jewish women with ovarian cancer. Thirty-six of the 242 women were the cases we identified in year one of the current study. We obtained BRCA1/2 genotyping data and reproductive data on these 242 women. We analyzed these data to determine the association between OC use and ovarian cancer risk in *BRCA1/2* carriers. Oral contraceptive use, childbearing and breastfeeding appear to protect *BRCA1* and *BRCA2* carriers from ovarian cancer, as they do for women in the general population. See attached manuscript for details.

REPORTABLE OUTCOMES:

A submitted manuscript detailing our findings is attached (Appendix 2). An abstract of this work was presented at the American Society of Human Genetics Annual Meeting [1].

CONCLUSIONS:

In conclusion, our initial findings suggest that both OC use and childbearing are protective in *BRCA1/2* carriers and non-carriers. We are currently working towards further analyzing this endpoint as well as the survival and treatment endpoint in our population.

We are concerned that due to delays at our NY site and the delay in obtaining IRB approval from Northwestern, we will be delayed in reaching our goals. While we are confident that we will complete the work tasks as outlined in the original proposal, we are concerned that we will not meet these goals on time. We anticipate requesting a 1 year no-cost extension from the DOD so that we complete the work as outlined in our proposal. We will work closely with the DOD to ensure the completion of this study.

REFERENCES:

1. Roxana Moslehi, Francesmary **Modugno,** Roberta B. Ness, Steven Narod.: Reproductive Factors and Ovarian Cancer Risk in Jewish BRCA1 and BRCA2 Mutation Carriers. In *Proceedings of the American Society of Human Genetics Annual Meeting.* San Diego, CA, October 2001. Also in *American Journal of Human Genetics* 69(4):274 Abstract 534.

APPENDICES:

IRB Approval letter from Northwestern University Hospital (D. Fishman, PI)
Draft of Manuscript

NORTHWESTERN

UNIVERSITY

July 5, 2002

David Fishman, MD
Obstetrics and Gynecology
Prentice 420
Chicago Campus

IRB Project # 0201-014

Re: **NU UPT02G1: Ovarian Cancer Risk and Survival in High-Risk Women**

Dear Dr. Fishman:

Full Institutional Review Board approval has now been granted for your project referenced above as of the IRB meeting 5/10/2002 for a one year period ending 5/10/2003. IRB approval includes approval of the protocol and it is noted that this study does not require a consent form.

IRB approval is given with the understanding that no changes may be made in the procedures to be followed until such modifications have been submitted to the IRB for review and have been given approval.

Any unanticipated problems involving risk to human subjects and any serious adverse effects must be reported promptly to the IRB.

If this is a sponsored project, please send a copy of this approval letter to the Office of Research and Sponsored Programs (ORSP). In addition if you make substantial changes to this project that may affect the contract please contact ORSP.

One month prior to the expiration of this approval, you will receive notification of the need for updated information to be used for the project's periodic review. All research involving human subjects and not specifically exempt from human subjects review must be reviewed and approved by the Institutional Review Board at least once a year. Information concerning implementation and results to date will be required at that time.

Sincerely,

Sigmund Weitzman pp.

Sigmund Weitzman, M.D.
Medical Director

Cc: Isabel Melendez
233 E. Erie St.-Suite #400
Chicago Campus

This Institution has an approved Federalwide Assurance with the Department of Health and Human Services: Assurance Identification Number FWA00001549.

Reproductive Factors and Ovarian Cancer Risk in Jewish BRCA1 and BRCA2 Mutation Carriers

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ABSTRACT

Objective: To determine whether oral contraceptive use, childbearing, breastfeeding and tubal ligation are protective against ovarian cancer in women carrying a *BRCA/2* mutation.

Methods: A case-only study of 242 Jewish women with invasive epithelial ovarian cancer. Women were genotyped for three Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2).* We obtained data on oral contraceptive (OC) use, childbearing, breastfeeding, gynecologic surgeries and other reproductive factors from each woman. We compared the frequencies of these risk factors in carriers and non-carriers using unconditional logistic-regression, controlling for other covariates.

Results: Among the 242 cases, 64 (26.4%) carried one of the *BRCA1* founder mutations, and 31 (12.8%) carried the *BRCA2* mutation. Although there were no differences in the percent of nulliparous women between carriers and non-carriers, parous *BRCA1* carriers reported fewer live births than non-carriers (average of 2.1 versus 2.5 live births, $P<0.02$). Carriers and non-carriers did not differ in their history of breastfeeding, or in their lifetime use of OC. *BRCA1* carriers began OCs at a later mean age (24.0 years versus 23.2 years of age, $P<0.04$) and were more likely to be recent users of OCs (average 19.6 versus 21.4 years since last use, $P=0.04$). *BRCA1* carriers were more likely than non-carriers to have had a tubal ligation (25.0% versus 10.2%, $P<0.01$).

Conclusions: OC use, childbearing and breastfeeding may protect *BRCA1* and *BRCA2* carriers from ovarian cancer.

Key Words: ovarian cancer, BRCA1, oral contraceptives, parity

INTRODUCTION

Mortality from invasive ovarian cancer is very high, with a five-year survival rate of approximately 30%[1]. Survival is better with early-stage disease, but the majority of patients present with metastatic disease[1]. To date, no effective early detection techniques have been identified and primary prevention represents an important opportunity for reducing ovarian cancer morbidity and mortality. Women with mutations in the cancer predisposing *BRCA1* and *BRCA2* genes have a lifetime ovarian cancer risk of 36%[2]. Using oral contraceptives, bearing children and breast-feeding have consistently been shown to reduce ovarian cancer risk among women in general[3,4]. Tubal ligation has also been shown to reduce ovarian cancer risk[3,5]. However, little is known about the impact of these factors on ovarian cancer risk in *BRCA1/2* mutation carriers. In a case-control study comparing 207 women with hereditary ovarian cancer to 161 of their unaffected sisters, OC use was less common among women with the disease[6]. This suggests that OC use may reduce the risk of ovarian cancer in women with a mutation in the *BRCA1* or *BRCA2* genes. However, a very recent case-control study of Israeli Jewish women found that the risk of ovarian cancer among carriers of a *BRCA1* or *BRCA2* mutation decreases with each birth but not with increased duration of use of oral contraceptives[7]. These conflicting data suggest the need to further investigate the potential of OCs as a chemopreventive agent among women with a *BRCA1/2* mutation.

In this study, we aimed to evaluate the potential benefit associated with OC use among women at high risk for ovarian cancer because they carry a mutated *BRCA1* or *BRCA2* gene. We also sought to determine the benefit or risk associated with other reproductive factors, including childbearing, breastfeeding, and tubal ligation in these women.

METHODS

Subjects

Because of the high prevalence of three *BRCA1/2* founder mutations among Ashkenazi Jewish women with invasive epithelial ovarian cancer[8], we limited our study to Jewish women with epithelial ovarian cancer and with no prior history of breast cancer. Data on subjects were pooled from four sources: two population-based case–control studies of epithelial ovarian cancer in the United States (100 cases)[9,10], a hospital-based study of Jewish women with epithelial ovarian cancer among 11 centers in North America and Israel (176 cases)[8], and a genetic counseling center in Chicago (14 cases). The Chicago clinic had been one of the sites for the hospital-based study, but the 14 incident cases included in this analysis were in addition to those participating in the original study. There was some overlap between cases included in the current study and those in the previous report of OCs and ovarian cancer in BRCA1/2 carriers[6] but this was less than 10%. Unfortunately, because subject links from the original studies to this study were not maintained, we were unable to identify which cases included in this study were also included in the previous report of OCs and ovarian cancer[6].

Moslehi et al.[8] (the hospital-bases study) classified a woman as Jewish if three out of four grandparents were Jewish. Questions about place of birth of parents and grandparents further identified Ashkenazi women in that study. In Lu et al. (one of the population-based studies),[10] a woman was considered to be Jewish if she indicated that her childhood religious upbringing was Jewish. For the other two sources of subjects, a woman was considered to be Jewish if she classified herself as Jewish on medical records.

Specific descriptions of each study methodology are provided in the original publications[8,9,9,10]. Briefly, Moslehi et al.[8] used medical records to identify 465 Jewish women with ovarian cancer. Of these, 80 women were dead, 33 women were found not to have invasive disease on pathology review, 98 women were unreachable, and 49 women refused to participate. The remaining 208 women completed an in-person interview and provided a blood sample. Ness et al.[9] identified all women age 20-69 diagnosed with ovarian cancer in the Delaware Valley between 1994 and 1998. Of the 957 eligible women, 69 were too ill to participate, 15 were untraceable, and 92 refused to participate. Fourteen physicians did not consent to their patients' participating, for a total of 767 eligible women who completed an in-person interview. For the study presented here, we used medical records to identify successfully the religious affiliation of 437 women, 46 of whom were Jewish, and we used banked pathology specimens to determine *BRCA1/2* carrier status. Lu et al.[10] used tumor registries to identify 1080 women with ovarian cancer in eastern Massachusetts and New Hampshire between May 1992 and March 1997. Of the 1080 women, 203 had died or were unreachable, 126 were

not contacted because their physician denied permission, 136 women declined participation, and 52 had non-epithelial ovarian cancer. The remaining 563 women were interviewed, during which time they provided a blood sample and answered questions about their childhood religious upbringing.

Each study obtained written informed consent from participants and was approved by the appropriate institutional review boards.

Exposure Information, BRCA1/2 Mutation Status and Data Quality

From each study source, data were requested on the use of oral contraceptives, including age at first and last use, and duration of use. Data were also obtained on number of live births, age at first and last live birth, and total duration of breastfeeding. We further requested information on other factors including age at menarche, body mass index, history of hysterectomy and history of tubal ligation. Because data on age at menopause and hormone replacement therapy were inconsistent among the studies, we were not able to include them in our analyses. We obtained details of tumor histology on all subjects, and we restricted our analyses to invasive ovarian cancers of the epithelial type. All data were checked for internal consistency and corrections or clarifications were requested from the original investigators when necessary.

All subjects were screened for the three Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2).* Mutation analysis was performed by the original study investigators using several established detection techniques, including

heteroduplex analysis, single-strand conformation analysis and allele-specific oligonucleotide hybridization. In addition, Moslehi et al[8] tested all subjects for mutations in exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* using the protein-truncation test[11]. Truncating mutations in these exons represent about 70% of the *BRCA1/2* mutations found to date[8]. No women from that study included in the analysis reported here were found to have any other *BRCA1/2* mutations. Regardless of the technique employed, all mutations were confirmed by direct sequencing of DNA. Non-carriers were defined as women with none of the three mutations. *BRCA1* carriers were defined as women with either the 185delAG or the 5382insC in *BRCA1*. Women with the 6174delT in *BRCA2* were defined as *BRCA2* mutation carriers.

All subject data submitted for the pooled analysis were anonymous. Approval for the pooled analysis was obtained from the University of Pittsburgh Institutional Review Board.

Study Design and Statistical Analyses

To determine whether carriers and non-carriers differed in OC use, parity, breast-feeding, and tubal ligation, we employed a case-only study design[12]. In a case-only study, cases with the genotype (carriers) form the "pseudo-cases" and cases without the susceptibility genotype (non-carriers) form the "pseudo-control" group. The two groups are compared with respect to the prevalence of each exposure. The odds ratio reflects the association between the exposure and the genotype (assuming independence of genotype and exposure). If this ratio is different from one, then the relative risk associated with the

exposure differs for carriers and non-carriers. For a protective factor such as OC use, childbearing and breastfeeding in ovarian cancer, an odds ratio (OR) greater than one indicates that the factor was more prevalent among the carriers ("pseudo-cases"); thus, the factor provides less protection to carriers than to non-carriers. Conversely, an OR of less than one indicates that the factor was less prevalent among the carriers, and suggests that the factor provides greater protection for carriers than for non-carriers.

To control for potentially confounding effects of other factors, we used unconditional logistic-regression analyses and included as covariates age at diagnosis and year of birth as continuous terms. Age at diagnosis was included in all models because univariate analyses showed a significant difference between carriers and non-carriers. Because the studies differed in the time period in which they were performed, year of birth was included in order to control for secular trends in OC use, parity and breastfeeding. However, there were no differences in results between analyses including year of birth and those excluding the variable. We therefore present the most parsimonious model in this paper. To check the reasonableness of pooling data from diverse sources, we calculated a Mantel-Haenszel test for heterogeneity for all major results. In none of the associations between BRCA status and reproductive factors did we find statistically significant heterogeneity among subject source. In addition, models that included a variable for study site did not differ in results from models excluding the variable; thus, the final models presented in this paper do not include a variable for study site. All analyses were performed with the STATA statistical software package (STATA Corporation, Release 5.0) and all *P* values given are from two-sided tests.

We analyzed all cases with complete exposure data. Because one of the parent studies[8] noted a difference in age at onset between *BRCA1* and *BRCA2* carriers, and because univariate analyses showed other differences in risk factors between *BRCA1* and *BRCA2* carriers for the entire study population, we analyzed the data for *BRCA1* and *BRCA2* carriers both jointly and separately.

RESULTS

A total of 290 cases of epithelial ovarian cancer in Jewish women were identified. Of these, 254 were confirmed to have invasive histology. Complete exposure data were obtained on all but 12 of these cases, for a total of 242 cases included in this analysis.

The characteristics of the 64 subjects with a *BRCA1* mutation, the 31 subjects with a *BRCA2* mutation, and the 147 non-carriers are presented in Table 1. As expected, *BRCA1* carriers with invasive tumors were diagnosed at a significantly earlier average age than non-carriers (51.2 versus 57.5 years, P=0.001). In contrast, *BRCA2* carriers were diagnosed at later ages than non-carriers (60.8 versus 57.5 years), although this difference was not significant. The difference in age at diagnosis between *BRCA1* carriers and *BRCA2* carriers, however, was significant (P<0.001).

Only 11.7% of non-carriers reported a family history of ovarian cancer, compared to 16.1% of *BRCA1* carriers (P>0.05) and 29.0% of *BRCA2* carriers (P=0.017 for comparison to non-carriers). Similarly, non-carriers were less likely to report a family

history of breast cancer (15.2% for non-carriers versus 22.6% for *BRCA1* carriers and 35.5% for *BRCA2* carriers). The difference between *BRCA2* carriers and the non-carriers was significant (P=0.011).

Table 2 compares reproductive factors among carriers and non-carriers. After adjusting for possible confounders, there were no significant differences between the groups for age at menarche, ages at first and last live birth, or breastfeeding. There was also no difference in the percent of nulliparous women between carriers and non-carriers. However, parous *BRCA1* carriers reported fewer live births than parous non-carriers. The average number of live births among parous women was 2.5 among non-carriers but only 2.1 among *BRCA1* carriers (P=0.02 adjusted for age at diagnosis, year of birth, tubal ligation and duration of OC use). Although parous *BRCA2* carriers also reported fewer live births than non-carriers, the difference between parous *BRCA2* carriers and non-carriers was not significant.

Interestingly, compared to non-carriers, *BRCA1* carriers were more likely to report having had a tubal ligation (25.0% versus 10.2%, P=0.003 adjusted for age at diagnosis, year of birth, number of live births and OC duration). *BRCA2* carriers were less likely to report a history of tubal ligation compared to non-carriers, but the difference was not significant. However, the difference between *BRCA1* and *BRCA2* carriers was significant (P<0.05).

We compared additional characteristics of oral contraceptive use between carriers and non-carriers (Table 3). No significant differences were found in ever use of OCs or in duration of OC use. However, *BRCA1* carriers were likely to have begun using OCs at a later mean age than non-carriers (24.0 versus 23.2 years of age, P=0.04 adjusted for age at diagnosis, number of live births, year of birth, tubal ligation and OC duration). *BRCA1* carriers were also more likely to report recent use of OCs. The mean interval from last use to diagnosis was 19.6 years for *BRCA1* carriers and 21.4 years for non-carriers (P=0.04 adjusted for age at diagnosis, number of live births, year of birth, tubal ligation and OC duration). The differences in age at first OC use or recent OC use between *BRCA2* carriers and non-carriers were not significant.

DISCUSSION

We pooled data on Jewish women with invasive ovarian cancer from four sources in order to determine whether the protection against ovarian cancer provided to women in the general population by OC use, childbearing, breastfeeding and tubal ligation applies equally to women carrying mutations in the *BRCA1* and *BRCA2* genes.

We found no difference in the percent of nulliparous women between carriers and non-carriers, although parous *BRCA1* carriers had experienced fewer live births than non-carriers. This suggests that bearing children is as protective against ovarian cancer among *BRCA1* carriers as it is among non-carriers. Our analyses showed a similar finding for parous *BRCA2* carriers, although the result failed to reach statistical significance, possibly due to the small number of *BRCA2* carriers in our study.

With regards to breastfeeding, we found no differences between *BRCA1/2* carriers and non-carriers. Thus, the effect of breastfeeding on ovarian cancer risk appears to be similar for both carriers and non-carriers.

We found that OC use also appeared to be equally protective for both carriers and non-carriers, confirming a previous report[6]. Notably, early OC use may provide greater protection to *BRCA1* carriers than to non-carriers, and recent use may not be as protective in *BRCA1* carriers as it is in non-carriers. We failed to demonstrate a similar association

between early OC use or recency of OC use for *BRCA2* carriers. Again, this may be due to differences in the effects of OC use in *BRCA2* carriers, or it may be due to the small number of *BRCA2* carriers in our study. Together, these results suggest that OCs may exert their effects early in a *BRCA1* carrier's reproductive life, whereas later exposure may not provide the same protection.

We further found that the protection associated with early OC use differed between *BRCA1* and *BRCA2*, although this difference may be due to the small number of *BRCA2* carriers. Notably, the direction of the ORs for the age and timing data among *BRCA2* carriers was opposite to that of the ORs for the *BRCA1* carriers, suggesting that the difference between the two groups may be real and not an artifact of sample size.

These results are in contrast to those of Modan et al.[7], who reported that the use of oral contraceptives provided no protection to Israeli Jewish *BRCA1/2* carriers. While we cannot exclude the possibility that our finding is due to chance, we believe that there are differences between the two studies that may explain these disparate findings. In particular, the duration and frequency of use of OCs were far less in the Israeli population than in the population studied here. Moreover, there may be differences in OC formulations between the two populations. In addition, as discussed below, the differences between the study designs (case-control versus case-only) may account for the different findings. Only further research will resolve the discrepancy.

Interestingly, *BRCA1* carriers were more likely to report having had a tubal ligation than non-carriers. Several studies have shown an association between tubal ligation and a reduction in ovarian cancer risk[5,13-15], although the exact mechanism remains unknown. Our results suggest that if the procedure does protect against ovarian cancer, it may not provide the same degree of protection to *BRCA1* carriers. This finding is in contrast to those of Narod et al. [16], who report a reduction in risk from tubal ligation among *BRCA1* carriers (OR=0.39, 95%CI= 0.21-0.63, adjusted for OC use, parity, history of breast cancer and ethnic group). Data from that study were obtained from a database containing information on women from high-risk families in Canada, the United States and the United Kingdom. The differences between that study and the results presented here may be due to differences in study populations (high-risk women with any *BRCA1/2* mutation versus Ashkenazi Jewish women with one of three mutations), study design (matched case control versus case only) or chance. In particular, the *BRCA1* gene has over 850 known mutations, and it is unknown whether risk factors for ovarian cancer vary by mutation type. Again, more research is needed to address these questions.

Care must be taken in interpreting our results. First, subjects were drawn from several sources. It is possible that the different study designs and data collection methods could have resulted in differences among the data sets that would affect our results. However, a likelihood ratio test indicated that the source of the data was not a significant factor in our analyses. Moreover, tests for heterogeneity between BRCA status and reproductive factors revealed no significant heterogeneity among subject source.

Second, we tested for a subset of mutations associated with ovarian cancer within a well-defined ethnic population. This raises the question of the generalizability of our results to the non-Jewish population or to women with other mutations.

Third, three of the four sources providing data for this study tested subjects for only the three mutations found in the Ashkenazim. Therefore, we may have missed some mutations and classified some carriers as non-carriers. However, the study providing the majority of cases[8] tested for most of the truncating mutations in *BRCA1* and *BRCA2* reported to date. Therefore, the occurrence of carrier misclassification would likely be small. Assuming that this misclassification is non-differential with respect to the exposures we examined, it would bias our results towards the null value.

About 40% of the cases included in this study were interviewed more than 1 year after their diagnoses. Women with a *BRCA1*or *BRCA2* mutation may have improved survival compared to women with nonhereditary ovarian cancer[17]. Therefore, it is possible that mutation carriers would be over represented among those interviewed more than a year after diagnosis. Indeed, among those women interviewed more than one year after diagnosis, 43% were *BRCA1/2* mutation carriers; among women interviewed within one year of diagnosis, only 36% carried a mutation. However, OC use, parity, breastfeeding and tubal ligation are not believed to be associated with cancer prognosis; therefore the

fact that our population included women interviewed more than a year after diagnosis should not affect our results.

Finally, our choice of a case-only approach has limitations that may have affected our findings. In particular, the case-only design assumes independence between the genetic marker and the environmental exposure[12]. However, it is often difficult to make this assessment, even in a large-scale study[7]. Hence, in the absence of such evidence, as is the case here, point estimates and confidence intervals must be interpreted cautiously. In particular, if there is uncertainty about the assumption that OC use and parity are independent of carrier status among Jewish women, then it is possible that the estimates reported here are less precise than the data suggest[18]. A case-control analysis would address this limitation. Unfortunately, because our data came from four sources with separate study designs, we lacked a valid control group to which we could compare the distribution of risk factors found among the different case groups. Moreover, because of the low prevalence of *BRCA1/2* mutations in the general population, it is unlikely that we would have had enough carriers in any control population to employ a standard interaction analysis.

In conclusion, our data suggest that using oral contraceptives, bearing children and breastfeeding provide protection from ovarian cancer in women with a *BRCA1* or *BRCA2* mutation. While the data presented here confirm previous findings[6], they stand in contrast to those reported recently by Modan et al[7] which suggested that OCs may not be protective in women with a *BRCA1* or *BRCA2* mutation. Moreover, our results contradict

the recent report that tubal ligation provides protection against ovarian cancer in *BRCA1* mutation carriers[16]. The disagreement between our study and these other studies on the protectiveness of OCs and tubal ligation indicate a substantial lack of clarity on how to counsel women at high risk for ovarian cancer. Further research is warranted.

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TABLE 1

Characteristics of BRCA1 and BRCA2 Carriers and Non-carriers

	BRCA- (n=147)	BRCA1+ (n=64)		BRCA2+ (n=31)	
Mean age at diagnosis (years)	57.5±12.5	**51.2±9.9**	**P=0.001**	60.8±11.3	P=0.20
Family history of ovarian cancer (%)	11.7	16.1	P=0.39	**29.0**	**P=0.017**
Family history of breast cancer (%)	15.2	22.6	P=0.20	**35.5**	**P=0.011**
Mean body mass index (kg/m2)	24.8±5.6	25.1 ±5.5	P=0.75	2.58±6.8	P=0.40
Mean year of birth	1936±12.8	**1941±9.9**	**P=0.005**	1932±12.1	P=0.14

*Plus-minus values are means ±SD. P-values are for comparison of carriers to non-carriers.

Missing data are as follows: 4 subjects (2 BRCA-, 2 BRCA1+), family history of breast and ovarian cancers; 1 BRCA2+ subject, BMI.

TABLE 2

Reproductive Characteristics of BRCA1 and BRCA2 Carriers and Non-carriers

	BRCA- (n=147)	BRCA1 /2+ (n=95) (all carriers combined)			BRCA1+ (n=64)			BRCA2+ (n=31)		
			Adj* OR	95% CI		Adj* OR	95% CI		Adj* OR	95% CI
Mean age at menarche (years)	12.7	12.5	0.93	0.75-1.11	12.6	1.01	0.81-1.26	12.3	0.79	0.60-1.06
Parous (%)[1]	84.4	82.1	0.89	0.42-1.87	76.6	0.67	0.29-1.52	93.6	2.50	0.54-11.68
No. of livebirths (%)[1]										
0	15.7	17.9	1.00		23.4	1.00		6.5	1.00	
1	10.9	14.7	1.15	0.44-3.05	14.0	0.80	0.26-2.36	16.1	3.61	0.60-21.57
2	37.4	43.2	0.99	0.44-2.20	45.3	0.84	0.34-2.07	38.7	2.29	0.45-11.48
≥3	36.1	24.2	0.59	0.24-1.41	17.2	0.34	0.12-0.97	38.7	2.27	0.44-11.48
Mean number of livebirths**,[1]	2.5	2.2	**0.70**	**0.49-0.99**	2.1	**0.61**	**0.39-0.95**	2.3	0.85	0.55-1.31
Mean age at first birth**	26.0	26.8	1.02	0.95-1.09	26.6	0.98	0.90-1.07	27.1	1.05	0.96-1.15
Mean age at last birth**	31.3	31.0	1.01	0.94-1.08	30.1	0.96	0.80-1.06	32.1	1.06	0.97-1.16
Mean time since first birth (years)**	32.7	29.4	0.98	0.92-1.06	26.9	1.02	0.94-1.11	33.6	0.95	0.86-1.04
Mean time since last birth (years)**	27.4	25.3	0.99	0.93-1.06	23.3	1.04	0.96-1.13	28.6	0.95	0.86-1.03
Breastfeeding (%)	32.0	31.6	1.09	0.61-1.97	34.4	1.36	0.68-2.73	25.8	0.70	0.28-1.72
Mean duration of breastfeeding (months)**	5.6	6.6	1.02	0.99-1.04	6.5	1.01	0.98-1.04	6.8	1.02	0.99-1.05
Tubal Ligation[2] (%)	10.2	19.0	2.32	1.06-5.11	25.0	**3.67**	**1.55-8.79**	6.5	0.65	0.14-3.16
Hysterectomy (%)	12.9	13.7	1.56	0.69-3.54	10.9	1.79	0.63-5.07	19.4	1.37	0.48-3.91

*Each row represents a separate model. All models were adjusted for age at diagnosis, number of live births (continuous variables) and OC use and history of tubal ligation (yes/no), except for those noted by ([1]), which were not adjusted for number of live births, and those noted by ([2]), which were not adjusted for tubal ligation. Odds ratios in bold are significant at the P<0.05 level.

**among women who had a live birth

Missing data is as follows: 1 BRCA- subject: age at first and last birth

TABLE 3

Oral Contraceptive Use Characteristics of BRCA1 and BRCA2 Carriers and Non-carriers

	BRCA- (n=147)	BRCA1/2+ (n=95) (all carriers combined)			BRCA1+ (n=64)			BRCA2+ (n=31)		
			Adj* OR	95% CI		Adj* OR	95% CI		Adj* OR	95% CI
Oral Contraceptive Use (%)[1]	39.2	49.5	1.21	0.67-2.17	56.3	1.29	0.66-2.51	35.5	1.11	0.44-2.76
Years of OC use (%)[1]										
Never	61.8	53.9	1.0		48.3	1.00		64.5	1.00	
<1	4.2	13.5	3.42	1.17-9.99	10.3	2.30	0.64-8.30	19.4	5.05	1.40-18.13
1-5	23.6	25.8	0.95	0.47-1.95	34.5	1.16	0.53-2.53	9.7	0.51	0.12-2.05
≥6	10.4	6.7	0.61	0.21-1.77	6.9	0.55	0.15-1.96	6.5	0.74	0.15-3.73
Mean duration of use (years)**, [1]	5.1	3.6	0.93	0.83-1.03	3.7	0.92	0.80-1.04	3.4	0.92	0.78-1.09
Mean age at first use**	23.2	23.9	1.11	0.99-1.24	24.0	1.14	1.01-1.30	23.6	0.96	0.80-1.16
Mean age at last use**	29.2	29.1	1.10	0.99-1.24	29.5	1.13	1.01-1.26	28.0	0.99	0.84-1.17
Mean time since first use (years)**	27.9	25.4	0.90	0.81-1.01	24.5	0.87	0.77-0.99	28.3	1.04	0.86-1.25
Mean time since last use (years)**	21.4	20.7	0.91	0.82-1.01	19.6	0.89	0.79-0.99	23.8	1.00	0.86-1.19

*Each row represents a separate model. Each model is adjusted for age at diagnosis, year of birth, number of live births, OC duration (continuous variables) and history of tubal ligation, except for those noted by ([1]), which were not adjusted for OC duration. Odds ratios in bold are significant at the P<0.05 level.

**among ever users